Note

HIV T cell vaccines, the importance of clades

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Abstract

Development of an HIV vaccine presents a formidable challenge. One of the unresolved, yet central issues is the importance of HIV variability. Here we argue that even with the recent focus on the induction of T cell-mediated immunity, HIV vaccines should match the local circulating HIV clades. Whether used alone or in a combination with vaccines eliciting HIV-neutralizing antibodies, efforts must be made to develop a T cell vaccine that stimulates a broad and long-lasting response. © 2002 Published by Elsevier Science Ltd.

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1. Introduction

It is likely that the HIV pandemic will only be controlled by a vaccine. Attempts to design vaccines that stimulate neutralising antibodies have been disappointing, though judgement should await the outcome of the phase 3 trials of gp120 to be reported in the next year. There has been a recent switch of attention to CD8+ T cell inducing vaccines in the hope that they can either prevent infection or control the virus more effectively. This article reviews the rationale behind this approach and asks whether clades will be important.

There is good evidence that CD8+ T cells (also known as cytotoxic T lymphocytes, CTL) are important in the control of HIV infection [1]. This T cell response appears shortly after infection and peaks a few days after the primary viraemia [2]. As the CD8+ T cells reach maximal numbers, upto 10% of all CD8+ T cells, the level of virus falls. However, if macaques infected with SIV are treated with anti-CD8 at this time, to delete or inactivate these cells in vivo, the viraemia does not decrease [3]. As HIV infection progresses the viraemia settles to the set point and the CD8+ T cell response falls to, normally 0.1–1.0% of all CD8+ T cells [1]. Evidence that the CD8+ T cells continue to control HIV comes from administration of anti-CD8 to chronically SIV infected macaques, which causes the virus level rises [3,4], and from the selection of virus escape mutants [5–7]. Ultimately the CD8+ T cell response fails

to control HIV, possibly because the loss of CD4+ T cell function and number, impairs the function of CD8+ T cells and thus their anti-viral properties [8].

HIV escape from CD8+ T cells has been extensively documented and appears to be a major feature of this infection [5]. This could lead to permanent changes in the virus if the mutant is transmitted to a person who shares the presenting HLA type [7]. This is obviously more likely for common HLA types. As these differ in different parts of the world, this process of immune escape and transmission could have contributed to the development of the clades; furthermore this is likely to be an ongoing process [1].

2. CD8+ T cell inducing vaccines

CD8+ T cells respond to peptide fragments of virus proteins that are presented by MHC class I molecules on the cell surface [9]. The peptides are derived from internal viral proteins that are degraded in the cytosol, predominantly by the proteasome, and then transported by the TAP transporter to the lumen of the endoplasmic reticulum where appropriate peptides bind to the HLA class I proteins [10]. The latter move to the cell surface where they are recognised by CD8+ T cells. This process has to be harnessed by a CD8+ T cell inducing vaccine [11]. Ideally a protein antigen has to be inserted into the cytosol. This can best be done by DNA transfection or by a carrier virus or other intracellular pathogen. Also dendritic cells can take up aggregated protein or particulate antigens into this pathway and stimulate CD8+ T cells [12]. These issues distinguish a

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T cell inducing vaccine from an antibody inducing vaccine and stress that different approaches are needed. A perfect vaccine may have to induce both T and B cell responses but it is likely that two vaccines could be mixed.

Plasmid DNA vaccines stimulate remarkably strong CD8+ T cell responses in mice, but may be less effective in macaques and humans [11]. The response can be enhanced by boosting with a virus vector expressing the same antigen. The combined effect can be 10 times the size of either alone in mice and macaques [13]. DNA priming is very focused and can be designed to present just the desired antigen, but the immune response tends to be weak. The recombinant virus gives a strong response presumably because of secondary signals, but alongside the inserted protein there may be more than 200 virus proteins. In a primary response the CD8+ T cells may respond to any of these, usually a few, and may often miss the insert. The DNA prime-boost may utilise the best properties of both component vaccines [11].

A key question is whether CD8+ T cells can actually protect against infection. Antibody to virus envelope can prevent a virus from entering cells. T cells cannot do this. The best they could do would be for the T cell to kill a virus infected cell before it can replicate to thousands of copies. This means the infection will occur, but will be terminated. There is no real evidence that HIV can be cleared in this way, but African sex workers who are uninfected despite very high exposure to HIV make CD8+ T cell responses [14]. These may be protecting them from actual infection.

There are better data from mice where pure CD8+ T cell responses induced by vaccines have been shown to protect against or decrease pathogen load of intracellular infections as diverse as herpes viruses [15], LCMV [16], *Listeria* [17] and *Plasmodium berghei* [13]. In some cases a single T cell epitope in the vaccine was sufficient to protect completely [13].

Recent studies in macaques have shown that vaccines that induce strong CD8+ T cell responses can partially protect against infection with SHIV 89.6 P [18,19]. This virus, an HIV/SIV hybrid, is particularly aggressive. It depletes CD4 T cells rapidly leading to early death in macaques. In animals vaccinated with DNA+ IL2, DNA+ recombinant (for the same Immunogen) MVA or DNA plus adjuvant, virus loads after infection were reduced by more than 1000-fold and the animals survived with normal CD4 counts [18,19]. Although not full protection, the virus doses used for challenge are much greater than human exposures and the virus used was very aggressive. However, it has been suggested that it might be easier to protect against this virus than a more insidious virus; this remains to be tested.

The above findings have encouraged a number of groups to test the hypothesis that vaccine-induced HIV specific CD8+ T cells could protect humans against HIV infection. Our study uses DNA to prime and recombinant MVA to boost; initial immune responses to the vaccine in phase 1 trials are encouraging. However, it will be some years before phase three trials can test efficacy.

3. Vaccine design

The greatest need for vaccines is in developing countries where non-B clades of virus predominate. In Kenya, 70% of viruses are A clade. Should a T cell inducing vaccine match the clade? There are pragmatic reasons why this should be the case. The people, scientists and authorities in developing countries are wary of western technology and, particularly in the AIDS field, the pharmaceutical industry. They may well not approve trials of a B clade vaccine for these reasons, which are understandable but not primarily scientific. A very positive message is given by taking a vaccine from a developed country to a developing country that is designed for the latter. Many countries still insist that the vaccine has been through phase 1 trials in the country of origin as well.

In fact there scientific reasons why a vaccine should be matched to the circulating clade. For a CD8+ T cell inducing vaccine, the T cells will respond to epitopes that are on average nine amino acids in length [20]. Extensive structural studies of HLA-peptide complexes show that of the 8–10 amino acids, three are normally involved in binding to the HLA molecule itself (anchor residues), three point towards the T cell receptor (TCR) (flag residues) and the remainder are in rather neutral positions (Fig. 1), Which amino acids are concerned varies with different HLA types, but the carboxy terminus residue is always an anchor and the penultimate side chain always points out towards the TCR.

The side chains of the anchor residues fit in to pockets in the peptide binding groove. These pockets are very sensitive

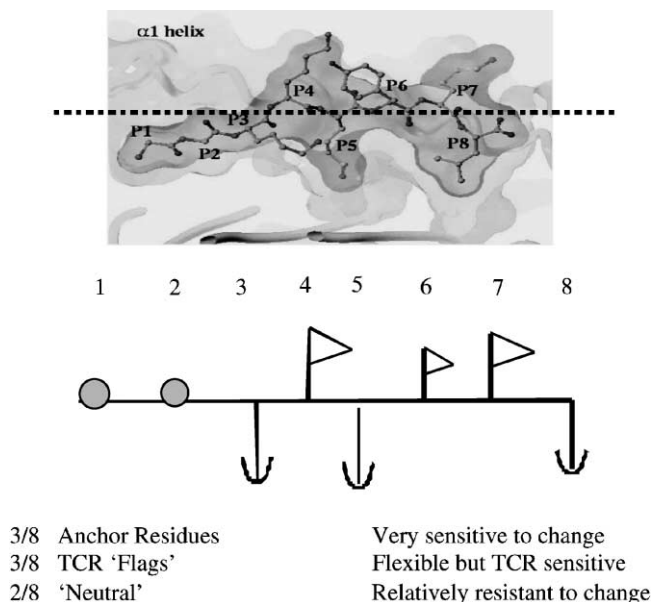| 3/8 | Anchor Residues | Very sensitive to change |
| 3/8 | TCR 'Flags' | Flexible but TCR sensitive |
| 2/8 | 'Neutral' | Relatively resistant to change |

Fig. 1. Distribution of anchor and flag residues in a peptide epitope binding to HLA B8. The upper part of the figure shows the structure of the HIV gag p17 epitope GGKKKYKL bound in the groove of HLA B8 [22], Above the dotted line are the side chains, positions 4, 6 and 7 that point out towards the T cell receptor (flags residues), below the line are those that bind to the B8 molecule (anchor residues). For a nonamer peptide there is usually an additional neutral residue.

to side chain differences. This is even true of conservative changes such as lysine to arginine or vice versa; in HLA B27, the B pocket will fit arginine but not lysine [21]. In HLA B8, both lysine and arginine fit into pockets but each shifts the position of other side chains in the peptide and even the α1 helix of the HLA molecule [22]. The carboxy terminal amino acid always binds in the F pocket and is restricted by other factors including the specificity of the proteasome proteases and the TAP transporter less than half of all amino acids are used and most alleles only allow three or four possibilities out of the 20. Thus, these three amino acids are very sensitive to changes.

The 'flag' side chains pointing towards the TCR usually include the penultimate amino acid, held in this orientation by the hydrogen bond between the carbonyl group of the last peptide bond and the conserved tryptophan at position 146 in the α2 helix. The side chain of amino acid four or five nearly always points towards the TCR and is important because this faces the central part of the TCR where the third hypervariable regions of the two chains, α and β meet. The side chain of the first amino acid also points upwards out of the groove but because this residue is deeply buried, the side chain may not be exposed. The side chains of residue seven, in a nonamer, is also often involved in TCR binding. Changes in these flag amino acid side chains can have variable effects. In general, the peptide still binds to the HLA molecule so is potentially immunogenic. However, T cell clones responding to one sequence may not recognise a mutant, although other clones might. If the mutation occurs in ongoing infection one might expect a new immune response to arise but for reasons not well understood this may not happen. This phenomenon is grandly termed 'original antigenic sin' and may arise if the new peptide stimulates the original T cells very weakly but enough to out compete a new primary T cell response. Another finding is that the alternative amino acid may antagonise the response to the original peptide [22]. This antagonism is well described experimentally and is theoretically important though hard to prove, The consequences are interesting because it would mean that the immune response to an epitope could be inhibited by a new variant. The infected person might then make a response to another epitope so that the immune response broadens. These issues are potentially very important for vaccines because a response to the invariant vaccine sequence might be badly undermined by infection with a virus that had an antagonist sequence at a key epitope.

In general three amino acids are relatively neutral in terms of HLA or TCR binding, although large changes, e.g. a phenylalanine for a glycine could disturb the position of the whole peptide in the groove and have effects on T cell recognition. Thus it can be argued that two-thirds of single amino acid changes will affect T cell recognition. This prediction is born out by a study in which each amino acid of a peptide epitope was changed to all 19 alternatives and each variant tested for recognition by T cell clones [23]. Only 59 of the 100 and 71 peptides were recognised, almost precisely one-third. Nearly all those not recognised were found at the three anchor and three flag residues.

4. Cross-clade recognition by CD8+ T cells

There are frequent reports of CD8+ T cells that cross-react between clades of HIV-1 but also descriptions of some that do not (e.g. [24]). Also there have been comparisons of sequences of particular epitopes across the clades [25]. However, we only know a small fraction, perhaps 1%, of all the possible epitopes presented by 500 HLA class one types, We propose that it is better to use the arguments above, which are based on firm structural data, on how peptides bind to HLA molecules, to estimate the likelihood of a vaccine sequence stimulating a CD8+ T cell response that will cross-react across clades.

The HIVA vaccine sequence described by Hanke and McMichael [11] has been used in phase 1 trials as DNA and inserted into modified vaccinia virus Ankara (MVA). It was designed as gag p17 and p24 consensus A clade sequence, aimed at trials and use in Kenya. It is 363 amino acids long and the C clade sequence differs in 22 of these, i.e. 1 in 16. If epitopes are evenly distributed across the protein, and there is no evidence to say otherwise, close to 1 in 2 epitopes will have one amino acid change. From the above argument, one-third of these would be recognised across the clades. Together with the half of the epitopes that are conserved this means that two-thirds of the epitopes in this vaccine can be expected to cross-react between A and C clades.

The HIVA construct is quite narrow encoding just gag p24 and p17 (plus 23 known conserved epitopes from other proteins as an epitope string). It is generally believed that the more virus proteins that are in the vaccine the broader the immune response will be. The HIVA vaccine appears to be more immunogenic than expected. Possible reasons are its small size as well as optimisation of codon usage, therefore we intend to make a second construct adding more virus proteins but focusing on immunogenic regions so as to keep size relatively small. The construct will include early expressed proteins, nef and tat as well as epitope rich regions of reverse transcriptase and gp41. This will be A clade and is less conserved across clades than HIVA.

5. Do clades matter for vaccine trials?

How important these differences are between clades depends on how many CD8+ T cell responses the vaccine stimulates. Because HIV usually stimulates broad responses to many epitopes there is a general expectation that a vaccine will do the same. However, the current candidate vaccines are cloned and nonreplicating so that they do not and cannot vary. In many virus infections in mice and humans, CD8+ T cell responses are narrow, specific for only a handful of epitopes. Therefore these vaccines might elicit narrow

responses to less than three epitopes, regardless of how many proteins are in the vaccine. The rather broader responses to HIV may well reflect the heterogeneity of the inoculating virus and the rapidity by which escape mutants can be selected by the early well-focused immune response. Once an escape occurs the immune response may focus on a new epitope, broadening the response.

If the vaccine stimulates a response to a few epitopes, which it could do even when the construct includes several virus proteins, the clade differences could be extremely important, restricting the use of the vaccine to clade-matched areas. A CTL response to a single epitope that differs in one amino acid would have a 66% chance of being ineffective for a C clade virus. However, if the response is broader there could be more effective coverage; a five epitope response would give 87% cross-reaction.

Another factor is whether a single epitope response is adequate, because the incoming virus might mutate and escape. This has been observed in a simian model [26]. Therefore a multiepitope response would be much preferable and this would be compromised if the infecting virus was from a different clade. All this emphasises that it is crucial to match the vaccine with the circulating virus clades for any vaccine trial, at least until we know how to elicit not only strong CD8+ T cell responses but broad responses to many epitopes.

6. Conclusion

Progress is being made in the design and testing of vaccines that stimulate CD8+ T cell responses and it is likely that this achievable with current approaches. Attention now needs to focus on the specificity and breadth of the elicited responses. It is likely that vaccine stimulated CTL responses will have to match the predominant clades of virus that circulate in the population for which the vaccine is designed. Even then the problem of virus variability may limit the effectiveness of the vaccine.

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